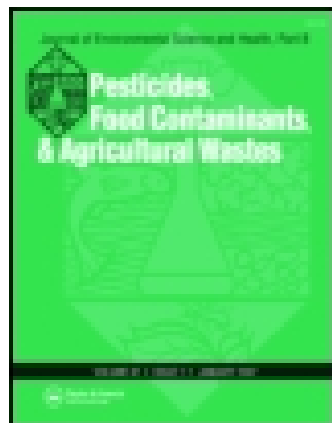


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# Degradation and sorption of imidacloprid in dissimilar surface and subsurface soils

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Degradation and sorption/desorption are important processes affecting the leaching of pesticides through soil. This research characterized the degradation and sorption of imidacloprid (1-[(6-chloro-3-pyridinyl)-methyl]-N-nitro-2-imidazolidinimine) in Drummer (silty clay loam) and Exeter (sandy loam) surface soils and their corresponding subsurface soils using sequential extraction methods over 400 days. By the end of the incubation, approximately 55% of imidacloprid applied at a rate of 1.0 mg kg<sup>-1</sup> degraded in the Exeter sandy loam surface and subsurface soils, compared to 40% of applied imidacloprid within 300 days in Drummer surface and subsurface soils. At the 0.1 mg kg<sup>-1</sup> application rate, dissipation was slower for all four soils. Water-extractable imidacloprid in Exeter surface soil decreased from 98% of applied at day 1 to >70% of the imidacloprid remaining after 400 d, as compared to 55% in the Drummer surface soil at day 1 and 12% at day 400. These data suggest that imidacloprid was bioavailable to degrading soil microorganisms and sorption/desorption was not the limiting factor for biodegradation. In subsurface soils >40% of <sup>14</sup>C-benzoic acid was mineralized over 21 days, demonstrating an active microbial community. In contrast, cumulative <sup>14</sup>CO<sub>2</sub> was less than 1.5% of applied <sup>14</sup>C-imidacloprid in all soils over 400 d. Qualitative differences in the microbial communities appear to limit the degradation of imidacloprid in the subsurface soils.

**Keywords:** degradation; sorption; insecticide; leach; partition; pesticide; subsoil; subsurface; transport.

## Introduction

Imidacloprid (1-[(6-chloro-3-pyridinyl)-methyl]-N-nitro-2-imidazolidinimine) is a systemic insecticide, which acts as an agonist of the nicotinyl acetylcholine receptor.<sup>[1,2]</sup> It is used as a seed dressing, soil treatment, and foliar treatment for controlling a variety of insects in a variety of crops. It is currently labeled for surface and subsurface application to soil, with effective soil application rates ranging from 0.3 to 50 mg kg<sup>-1</sup> depending on the target pest.<sup>[3–5]</sup>

Relatively little information on the fate of imidacloprid in soil has been published. It has a relatively very long half-life in soil; surface soil dissipation half-lives (DT<sub>50</sub>) in field experiments under various cropped and agricultural conditions ranged from 40 to 190 d.<sup>[6–8]</sup> Half-lives of 48 d have been reported when vegetation is present<sup>[8]</sup>; and 180 or 190 d in nonvegetated soil.<sup>[8,9]</sup> In laboratory studies at normal

agricultural rates, DT<sub>50</sub> values have been comparable to the shortest DT<sub>50</sub> observed in field studies<sup>[10]</sup>, however, at higher termiticidal application rates, extrapolated DT<sub>50</sub> values ranged from 5 to 10 months.<sup>[5,11]</sup>

Persistence in soil can result from decreasing bioavailability over time or from low activity of the microbial community towards the pesticide, or from a combination of these factors. Bioavailability of a compound indicates the degree to which a microorganism, plant, invertebrate, or vertebrate is able to take up and metabolize the insecticide or chemical.<sup>[12]</sup> Bioavailability is difficult to quantify and is partly dependent on the model organism. Currently, there is no standard method to determine bioavailability of a pesticide. Also, bioavailability of a pesticide may change over time; the longer a compound remains in the soil the more likely it is to be less bioavailable.<sup>[13]</sup>

The decreased rate of imidacloprid degradation in soil with the addition of organic amendment was attributed to increased sorption resulting in decreased bioavailability.<sup>[7]</sup> In general, it has been shown that soil sorption of imidacloprid increases in soils with increasing organic carbon (OC) and clay contents.<sup>[14–16]</sup> Sorption to isolated clays and organoclays has also been shown.<sup>[17,18]</sup> As for

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many pesticides, desorption of imidacloprid is hysteretic in both surface and subsurface soils<sup>[14,16]</sup>, possibly due to irreversible sorption on different soil components.<sup>[19]</sup> Increased sorption and decreased desorption with time would account for the increased sorption observed for aged residues<sup>[9,20]</sup> and make biodegradation more difficult.

Although imidacloprid is water soluble (0.51 g L<sup>-1</sup>), it does not tend to leach below the surface soil when surface-applied.<sup>[6,7]</sup> Leaching has been observed in greenhouse soils,<sup>[21]</sup> in field soils following the application of imidacloprid via drip irrigation,<sup>[22]</sup> and by preferential transport in cracking soil.<sup>[23]</sup> Imidacloprid applied by subsurface drip chemigation leached to at least a 150 cm depth in field trials.<sup>[24]</sup> The U.S. EPA reports that imidacloprid has been detected in groundwater in areas vulnerable to leaching.<sup>[25]</sup>

There is no information available on the degradation and limited information on sorption of imidacloprid in subsurface soils.<sup>[16]</sup> Knowledge of pesticide degradation and sorption/desorption in subsurface soils is needed for assessments of the bioavailability and leaching potential of these chemicals and for assessing risk of ground water contamination by pesticides. The objective of this study was to determine imidacloprid bioavailability, as characterized by its degradation and sorption in surface and subsurface soils. To accomplish this, a soil incubation study was conducted to relate long-term persistence of imidacloprid to water-extractable and solvent-extractable concentrations in soil.

## Materials and methods

### Chemicals and soils

An analytical standard of imidacloprid (1-[(6-chloro-3-pyridinyl)-methyl]-*N*-nitro-2-imidazolidinimine) (96.9% pure) was obtained from Bayer Corporation. Radiolabeled imidacloprid (<sup>14</sup>C-methylene-imidacloprid, 32.1 mCi mmol<sup>-1</sup>, 99.9% pure) was also obtained from Bayer Corporation. <sup>14</sup>C-ring-labeled benzoic acid (specific activity: 0.05 mCi mmol<sup>-1</sup>) was obtained from Sigma (St. Louis, MO).

Agricultural soils from field sites near Oxford, Indiana (Drummer silty clay loam (SiCL): fine-silty, mixed, superactive, mesic; Typic Endoaquolls) and Fresno, California (Exeter sandy loam (SL): fine-loamy, mixed, superactive, thermic; Typic Durixeralfs) were sampled at surface and subsurface depths. The Drummer soil never had imidacloprid applied to it and had no pesticide application in the year it was collected. The Exeter soil also had never been exposed to imidacloprid and had no pesticide application history in the five years prior to its collection. Soils from the following depths were collected: Drummer and Exeter surface soils, 0–15 cm; Drummer subsurface soil, 15–76 cm; and Exeter subsurface soil, 46–61 cm. Soils were removed from the field site with hand shovels, placed in water impermeable bags and shipped to the National Soil Tilth Labo-

**Table 1.** Selected soil physicochemical and biological properties of Drummer and Exeter soils.

Soil	Depth (cm)	Water Content (% at -50 kPa)	N (%)	OC (%)	Microbial Biomass (μg C g <sup>-1</sup> )	pH
Exeter sandy loam	0–15	5.86	0.03	0.21	43.2	4.6
	46–61	6.18	0.02	0.11	10.2	5.6
Drummer silty clay loam	0–15	36.1	0.36	4.49	704.7	5.6
	15–76	27.3	0.08	0.81	104.7	5.9

OC: organic carbon.

ratory, Ames, IA. Soils were stored at 7°C before the study. Moist soils were passed through a 4-mm sieve and soil moistures were taken prior to treatment with imidacloprid.

Soil OC and total nitrogen were determined using a Carlo-Erba NA 1500 NCS elemental analyzer (Haake Buchler Instruments, Paterson, NJ). Total soil microbial biomass C was measured by the fumigation-extraction method with analysis of the extracts on a Dohrmann DC-180 carb on analyzer (Rosemount Analytical Services, Santa Clara, CA).<sup>[26]</sup> Soil microbial biomass was measured as the difference in extractable C between the fumigated and non-fumigated samples and calculated using the correction factor *k* (*k* = 0.33).<sup>[27]</sup> Selected physicochemical (Midwest Laboratories, Inc., Omaha, NE) and biological properties are listed in Table 1.

### Soil treatment

Soil aliquots of 30 g (dry weight basis) in beakers, were treated in triplicate with either 3 μg <sup>14</sup>C-methylene-imidacloprid or 30 μg non-radiolabeled imidacloprid, in 1 mL acetonitrile (ACN), giving a final concentration of either 0.1 mg kg<sup>-1</sup> or 1.0 mg kg<sup>-1</sup>. Treated soils were thoroughly mixed and then moistened with ultrapure water to bring them to -50 kPa water potential, which is approximately 75% of field capacity. Soils were thoroughly mixed again in order to ensure uniform distribution of the water and imidacloprid. Each beaker with soil was placed inside a 946-ml Mason jar, along with a vial containing 10 mL of 0.5 M NaOH. The jars were sealed tightly and stored at 25°C in the dark for up to 400 days. The jars were opened weekly for surface soils and biweekly for subsurface soils in order to maintain aerobic conditions, and NaOH containing vials were replaced with fresh solution. The NaOH that was removed from the vials was analyzed for <sup>14</sup>CO<sub>2</sub> by mixing 1 mL aliquots of NaOH with 5 mL liquid scintillation cocktail and measuring radioactivity by liquid scintillation spectroscopy (LSC). The NaOH containing vials were replaced each time with fresh solution. Soil water contents were determined gravimetrically and replenished, if needed.

In order to assess biological activity of the soil microbial communities, additional samples of these soils were treated

with  $^{14}\text{C}$ [ring] benzoic acid at  $25 \text{ ug g}^{-1}$  soil. Soil incubations and  $^{14}\text{CO}_2$  trapping was performed as described above for 30 d.

### Soil extraction and analyses

Soil samples were removed from the beakers after either 1, 28, 84, 112, 308, or 400 days of incubation, and put into Whirlpack bags and stored in a freezer until they were extracted. Soils treated with imidacloprid at  $1.0 \text{ mg kg}^{-1}$  were extracted using an Automated Solvent Extractor (ASE) (Dionex Corp., Sunnyvale, CA). The 30-g soil samples were divided in half and 15 g were put into each of two stainless steel cells for extraction along with 2 g of hydro-matrix (Varian Corporation, Lake Forest, CA). Each cell was sequentially extracted with 0.01 M  $\text{CaCl}_2$ , ACN, and a mixture of 10% 0.02 M phosphoric acid: 90% ACN and ethyl acetate (80:20 v:v). Conditions for the ASE were as follows: preheat 5 min, static 5 min, flush 60%, purge 60 s, 3 cycles, pressure 2050 psi, and  $75^\circ\text{C}$ . A total of approximately 40 mL of extract for each of the three solvent systems was collected per vial. Preliminary studies showed total recoveries were greater than 86% for all soils using this method. These recoveries are comparable to those of soils extracted using 80% ACN: 20%  $\text{H}_2\text{O}$  in an ultra-sonic bath.<sup>[28]</sup>

The combined aqueous  $\text{CaCl}_2$  fractions were passed through preconditioned C-18 solid phase extraction (SPE) cartridges.<sup>[29]</sup> Slight alterations of the method were made by eluting the cartridges with 5 mL methanol and after evaporating the methanol, dissolving the concentrated samples in 83% ACN:17%  $\text{H}_2\text{O}$ . The ACN and acid fractions were concentrated under nitrogen gas to volumes between 2 and 4 mL. Extracts were analyzed using a liquid chromatograph-mass spectrometer (LC-MS) (Waters Micromass ZMD coupled to a Waters Alliance 2690 HPLC, Milford, MA) under the following conditions: Zorbax C-8 column ( $2.1 \text{ mm} \times 15 \text{ cm}$ ),  $10 \mu\text{L}$  injection, flow rate  $0.2 \text{ mL min}^{-1}$ , and a gradient of ACN and 1% formic acid/water starting at 85% ACN at 0 min, decreasing to 70% ACN after 3 min, to 60% ACN after 6 min, to 50% ACN after 12 min, to 20% ACN after 15 min, and then back to 85% ACN after 20 min.

Soils treated with  $^{14}\text{C}$ -imidacloprid at  $0.1 \text{ mg kg}^{-1}$  were sequentially extracted by shaking with solvents as previously reported.<sup>[20,30]</sup> In brief, soils were first extracted by shaking with 0.01 M  $\text{CaCl}_2$ . After the supernatant was removed for analysis, remaining soil was then extracted by shaking with acetonitrile:water. The supernatant was removed and saved for analysis. The supernatants were analyzed for total radioactivity by LSC and for imidacloprid and selected metabolites by high performance liquid chromatography (HPLC) and LSC as previously reported.<sup>[20,30]</sup>

### Data analyses

The distribution coefficient  $K_d$  is defined as the partitioning of pesticides between sorbed and soluble phases. These co-

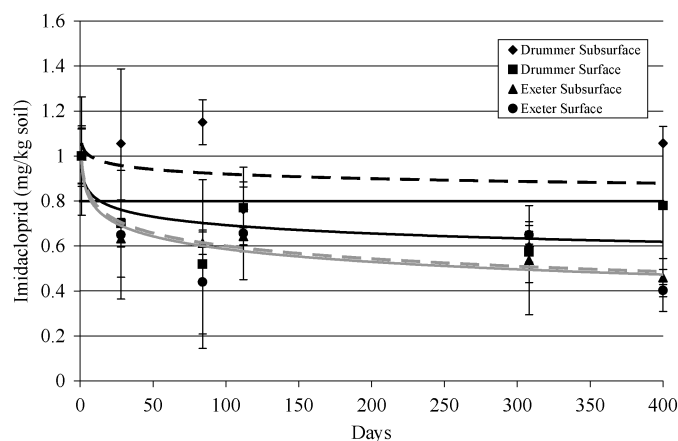
efficients have been normally determined using batch slurry techniques, where sorbed amounts are not directly determined, but calculated from the pesticide lost from solution. We present an alternative estimate of  $K_d$  where the aqueous  $\text{CaCl}_2$ -extractable imidacloprid represents the solution concentration ( $C_e$ ) in batch slurry sorption-desorption studies, the ACN-extractable imidacloprid represents the sorbed concentration ( $C_s$ ), and  $K_d = C_s/C_e$ .

## Results and discussion

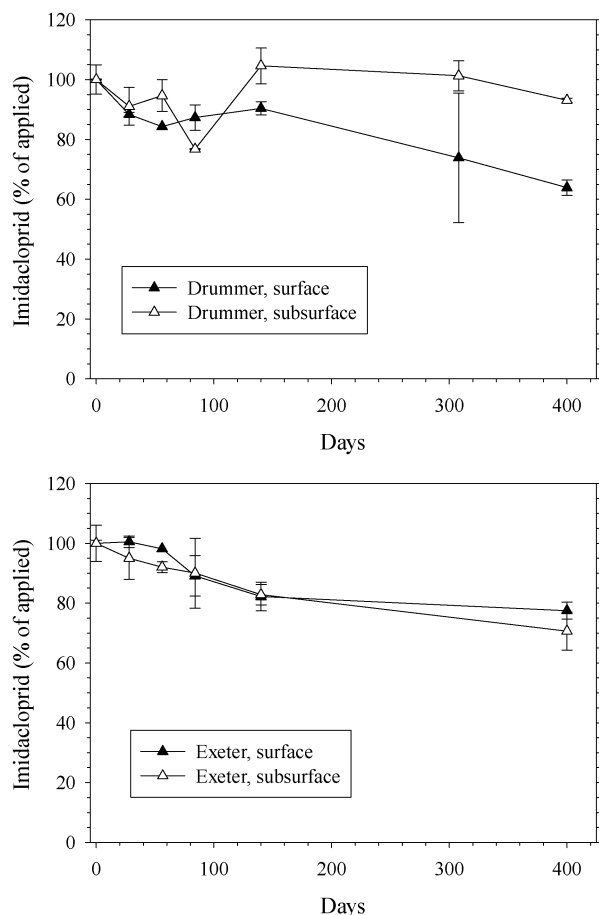
### Degradation

Imidacloprid degraded slowly in both surface and subsurface soils during the 400-d incubation (Fig. 1). By the end of the incubation, approximately 55% of imidacloprid applied at a rate of  $1.0 \text{ mg kg}^{-1}$  degraded in the Exeter surface and subsurface soils. In Drummer surface soil, nearly 40% of applied imidacloprid degraded within 300 d, and at 400 days two of the three replicates had 35% or more of the imidacloprid degraded. At the  $0.1 \text{ mg kg}^{-1}$  application rate, approximately 23% of applied imidacloprid degraded during the 400-d incubation in the Exeter surface soil and 29% in the subsurface soil (Fig. 2). In contrast, in the Drummer soil, 36% of applied imidacloprid degraded in the surface soil, whereas there was no degradation in the subsurface soil after 400 d. The initial application of imidacloprid in acetonitrile may have slowed the initial degradation, but rapid degradation of acetonitrile by a wide variety of microorganisms has been reported and any effects would likely be of short duration.<sup>[31]</sup>

For the soils that exhibited loss of imidacloprid, the degradation appeared to be biphasic. Imidacloprid concentration decreased rapidly during the first 28 days followed by a comparatively slower decrease thereafter. For instance, in the Exeter surface soil, of the applied  $0.1 \text{ mg kg}^{-1}$ , 18%



**Fig. 1.** Degradation of imidacloprid applied at  $1.0 \text{ mg kg}^{-1}$  to Drummer (black lines) and Exeter (gray lines) soils over 400 days. Surface soils are represented by solid lines and subsurface soils by dashed lines.



**Fig. 2.** Degradation of imidacloprid applied at  $0.1 \text{ mg kg}^{-1}$  to Drummer and Exeter soils over 400 days.

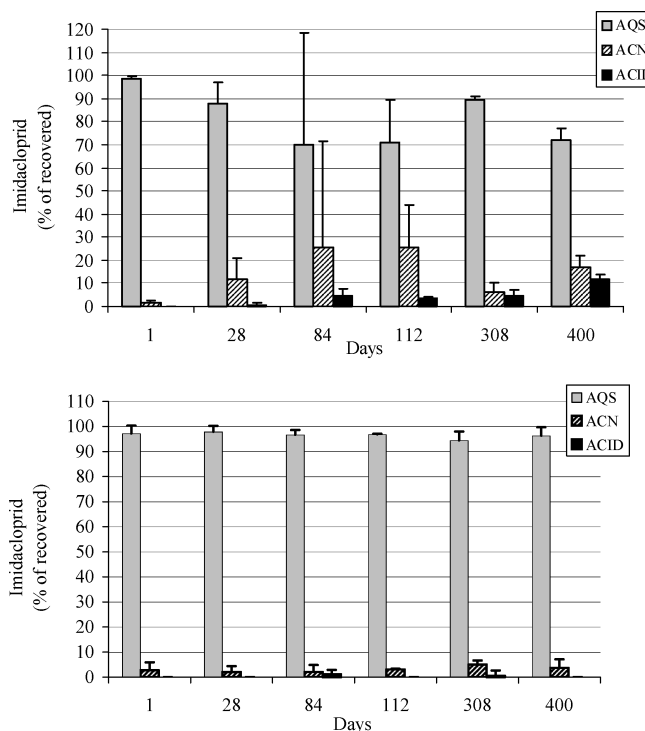
degraded in the first 140 days and 12% degraded during the final 240 d. Similar results were observed in the Exeter subsurface soil; 17% degraded in the first 14 d and 13% in the last 240 d. This is in contrast to other field and laboratory degradation studies in which first-order half-lives ranged between 48 d and 190 d.<sup>[8]</sup> The combination of variability and the biphasic pattern of degradation prevented an adequate assessment of degradation kinetics. Best-fit trend lines (Fig.1) were obtained by least-squares regression using the model,  $y = a[\ln(x)] + b$ , where  $y$  is imidacloprid concentration and  $x$  is days of incubation. Clearly,  $DT_{50}$  times are approximately 400 days or longer.

Imidacloprid is subject to microbial degradation with production of both the urea (1-[(6-chloro-3-pyridinyl)methyl]-2-imidazolidinone) and guanidine (1-[(6-chloro-3-pyridinyl)methyl]-4,5-dihydro-1*H*-imidazol-2-amine) metabolites in liquid culture.<sup>[32]</sup> The [ $^{14}\text{C}$ -methylene]-imidacloprid-guanidine and imidacloprid-urea metabolites were identified on multiple sampling dates in both surface and subsurface soils (data not shown). Recovery of imidacloprid-urea metabolite by the water and acetonitrile extracts averaged (over all sample dates) between 5.8 and 11.3% of applied imidacloprid. Imidacloprid-guanidine in

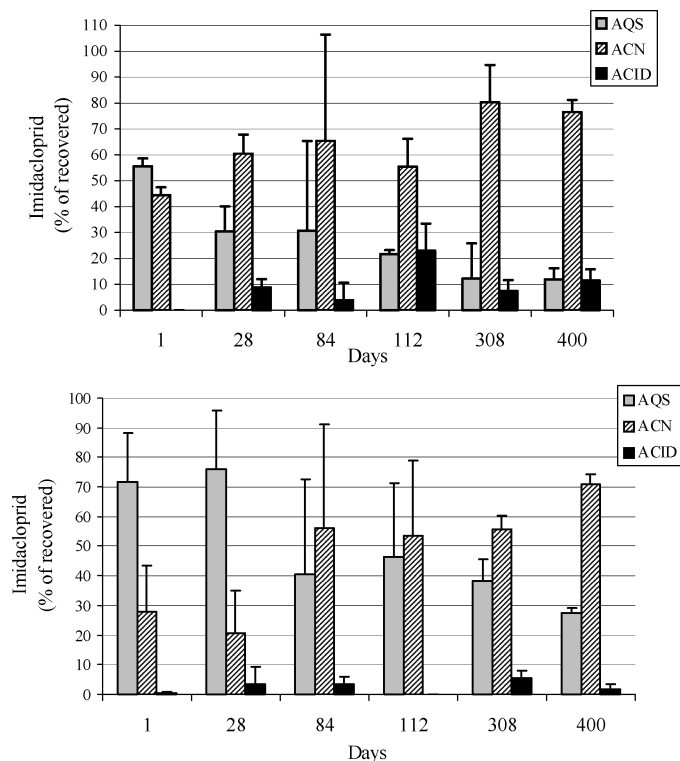
these extracts averaged between 2.8 and 8.7% of the applied imidacloprid. There was nearly equal amounts of metabolites in the water extracts as in the acetonitrile extracts and these ratios did not change over time. Very little ( $<1.7\%$  of applied) mineralization of the  $^{14}\text{C}$ -methylene-labeled imidacloprid was observed in both surface and subsurface soils (data not shown). Bound residue (non-extractable  $^{14}\text{C}$ ) increased over time reaching a final level of 10% in the Exeter surface soil, 5.2% in the Exeter subsurface soil, 11.5% in the Drummer surface soil, and 6% in the Drummer subsurface soil.

To characterize imidacloprid availability, soils were sequentially extracted with 0.01 M  $\text{CaCl}_2$ , ACN, and acidic ACN/ethyl acetate. Aqueous-extractable imidacloprid is assumed to be readily available for transformation by microorganisms. ACN-extractable imidacloprid (sorbed fraction) is assumed to be slowly available for microbial use,<sup>[13,33]</sup> and the acid-extractable (strongly sorbed fraction) may not be available. The availability can be influenced by soil properties, with higher OC and clay soils tending to have less imidacloprid available to microorganisms.<sup>[14,15]</sup>

When applied at  $1.0 \text{ mg kg}^{-1}$ , it appears that more imidacloprid was available in Exeter soil throughout the 400-d incubation compared to Drummer soil. Water-extractable imidacloprid in Exeter surface soil decreased from 98% of applied at Day 1 to  $>70\%$  of the imidacloprid remaining after 400 days (Fig. 3), as compared to 55% in the Drummer



**Fig. 3.** Imidacloprid recovered by sequential aqueous (AQS), acetonitrile (ACN) and acid (ACID) extractions from Exeter surface (top) and subsurface (bottom) soils expressed as a percentage of total imidacloprid recovered.



**Fig. 4.** Imidacloprid recovered by sequential aqueous (AQS), acetonitrile (ACN) and acid (ACID) extractions from Drummer surface (top) and subsurface (bottom) soils expressed as a percentage of total imidacloprid recovered.

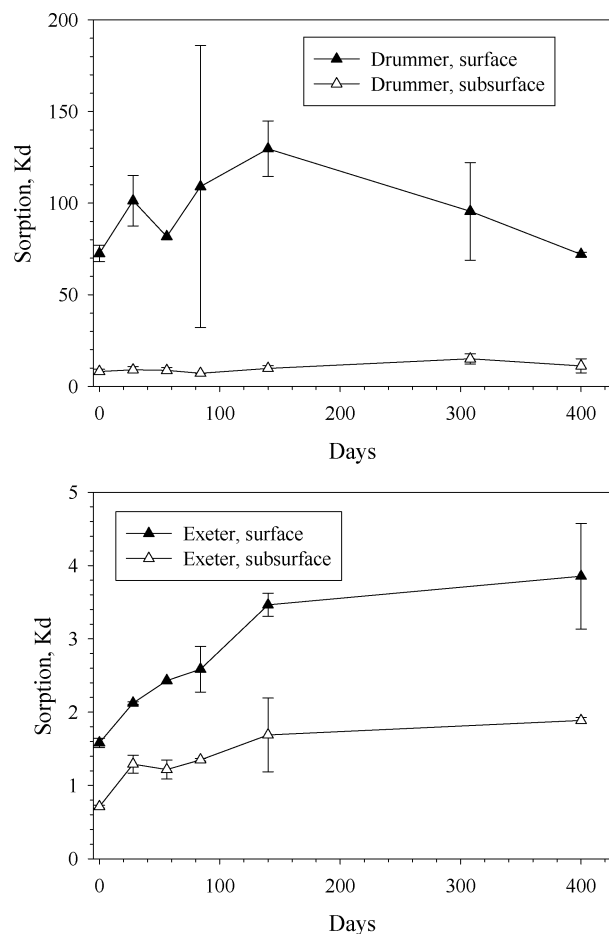
surface soil at Day 1 and 12% at Day 400 (Fig. 4). This is likely due to the higher organic carbon content in the Drummer soil (Table 1), which would increase sorption of imidacloprid.<sup>[15]</sup> Water-extractable imidacloprid decreased in the Exeter surface soil through day 84 then remained constant; in contrast, there was very little change in the subsurface soil (Fig. 3). In the Drummer surface soil, available imidacloprid slowly decreased throughout the 400-d incubation, with the greatest absolute decrease during the first 28 d. In subsurface Drummer soil, available imidacloprid decreased at day 84 and then remained constant (Fig. 4). Similar results were obtained for imidacloprid applied at 0.1 mg kg<sup>-1</sup> (data not shown).

Distribution coefficients ( $K_d$ ), or partitioning of pesticides between sorbed and soluble phases, have been used to characterize differences in pesticide availability between soils. These coefficients have been normally determined using batch slurry techniques, where sorbed amounts are not directly determined, but calculated from the pesticide lost from solution. In contrast, we used the amounts of imidacloprid extractable by aqueous CaCl<sub>2</sub> as the solution concentration ( $C_e$ ), and the ACN-extractable as the sorbed concentration. The HCl extractable residues are tightly bound and are not likely to desorb from the soil and thus were not considered in the sorption partition coefficient. For imidacloprid applied at 1.0 mg kg<sup>-1</sup>,  $K_d$  values calcu-

lated for Day 1 were 0.02 and 0.07 mL g<sup>-1</sup> for the Exeter surface and subsurface soils, respectively.  $K_d$  values increased after the 400-d incubation to 0.55 and 0.10 mL g<sup>-1</sup> for surface and subsurface soils, respectively. In Drummer soil,  $K_d$  values increased by a factor of 6–8 in the surface soil (1.9 to 15.4 mL g<sup>-1</sup>) and subsurface soil (1.0 to 6.2 mL g<sup>-1</sup>).

Drummer and Exeter surface and subsurface soils were much more sorptive at the lower rate of 0.1 mg kg<sup>-1</sup> imidacloprid (Fig. 5), as compared to the higher rate of imidacloprid. Such results are similar to what has been observed previously.<sup>[14]</sup> As a result of the high initial sorption, the changes in sorption with incubation time were not as great in the Drummer soil as those for the less sorptive Exeter soils. In Drummer surface and subsurface soils,  $K_d$  increased by a factor of <2 between 0 and 300 d, and factor of ~2.5 in the Exeter surface and subsurface soils (Fig. 5). The greater relative sorption at low concentrations would likely slow leaching of imidacloprid because of the low concentrations leaving the surface soil.

Aging of pesticides tends to decrease their bioavailability to microorganisms and higher organisms.<sup>[13]</sup> This decrease in bioavailability occurs by the fairly rapid sorption of the



**Fig. 5.** Change in sorption of imidacloprid applied to Drummer and Exeter soils at 0.1 mg kg<sup>-1</sup> over 400 days.

pesticide to external soil surfaces and then a slower partitioning of the pesticide into the inner soil surfaces and organic matter. This is referred to as a biphasic model of sorption.<sup>[12,13]</sup> Our results are generally consistent with this model of pesticide behavior.

Generally, microorganisms in surface soils are able to degrade pesticides more readily than in subsurface soils due to their higher populations, greater metabolic activity, and greater diversity. Pesticides such as alachlor, metribuzin, fluometuron, and 2,4-D all degrade more slowly in subsurface than surface soils.<sup>[34–37]</sup> In the Drummer subsurface soil, degradation of imidacloprid was slower than in the Drummer surface soil, however no difference was observed between Exeter surface and subsurface soils (Fig. 1). The distribution of imidacloprid in the water-, acetonitrile-, and acid-fractions was similar in the Exeter surface and subsurface soils, although more water-extractable was found in the Exeter subsurface. In the Drummer soils, after 112 d, both the surface and subsurface soils showed a similar pattern, with more imidacloprid found in the acetonitrile-extractable fraction (Fig. 3). Only 10% of the imidacloprid was degraded in the Drummer subsurface soil, but 25% of the imidacloprid was still water-extractable after 400 days, suggesting that bioavailability was not limiting, but rather the activity of degrading microorganisms.

Microbial biomass in these two soil profiles was lower in subsurface soils than in surface soils (Table 1) and there was greater microbial biomass in the Drummer soil than in the Exeter soil. Benzoic acid is quickly metabolized by microorganisms and we used mineralization of this compound as an index of microbial activity. Benzoic acid was mineralized very quickly by the Drummer surface soil with more than 25% mineralized within 24 h, compared to the Drummer subsurface soil which took more than 3 d to mineralize the same amount of chemical (Fig. 6). In contrast, benzoic acid was mineralized in the low biomass Exeter soils after longer

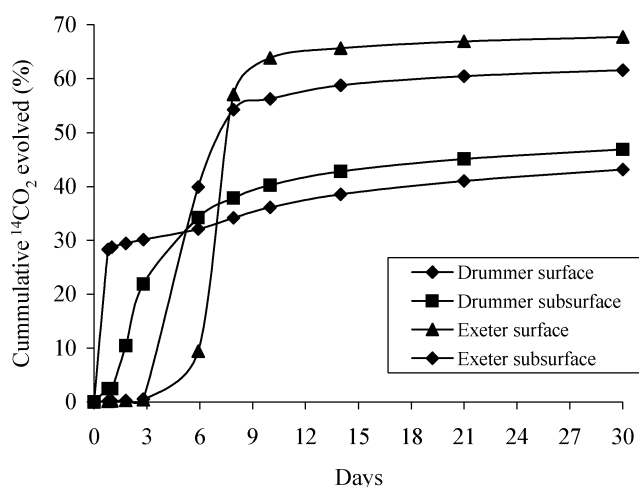


Fig. 6. Cumulative mineralization of <sup>14</sup>C-benzoic acid in Drummer and Exeter surface and subsurface soils.

lag periods than the Drummer soil. These results demonstrate a greater level of microbial activity in the Drummer soils, but also show some metabolic activity in the Exeter soils.

While microbial biomass and benzoic acid mineralization show relative levels of microbial population and activity in these soils, pesticide degradation is conducted by the activity of specific populations that contain the requisite genes and enzymes required for degradation.<sup>[38]</sup> Imidacloprid persistence in soil is likely controlled by the population and activity of imidacloprid-degrading microorganisms in these soils, in combination with bioavailability. In view of the fact that three of the four soils had a high concentration of imidacloprid present in the water-extractable fraction after a 400-d incubation, it seems reasonable to assume that these imidacloprid quantities are bioavailable. Despite these results, degradation was still limited in these soils. This indicates that the microorganisms necessary for imidacloprid degradation are either present in low populations or are limited in their activity.

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